Regulation of fatty acid uptake and metabolism in L6 skeletal muscle cells by resistin

Rengasamy Palanivel, Gary Sweeney*

Department of Biology, York University, Toronto, Ont., Canada M3J 1P3

Received 22 July 2005; accepted 3 August 2005

Available online 19 August 2005

Edited by Robert Barouki

Abstract **Resistin has been proposed as a potential link between obesity and insulin resistance. It is also well established that altered metabolism of fatty acids by skeletal muscle can lead to insulin resistance and lipotoxicity. However, little is known about the effect of resistin on long chain fatty acid uptake and metabolism in skeletal muscle. Here we show that treating rat skeletal muscle cells with recombinant resistin (50 nM, 24 h) decreased uptake of palmitate. This correlated with reduced cell surface CD36 content and lower expression of FATP1, but no change in FATP4 or CD36 expression. We also found that resistin decreased fatty acid oxidation by measuring $^{14}CO_2$ production from [1-^{14}C] oleate and an increase in intracellular lipid accumulation was detected in response to resistin. Decreased AMPK and ACC phosphorylation were observed in response to resistin while expression of ACC and AMPK isoforms was unaltered. Resistin mediated these effects without altering cell viability. In summary, our results demonstrate that chronic incubation of skeletal muscle cells with resistin decreased fatty acid uptake and metabolism via a mechanism involving decreased cell surface CD36 content, FATP1 expression and a decrease in phosphorylation of AMPK and ACC.**

1. Introduction

There has been considerable debate as to whether resistin [1] may play a role in the etiology of insulin resistance and diabetes in obesity [2]. However, an increased plasma resistin concentration was observed in serum of obese [3] or type 2 diabetic individuals [4] and thiazolidinedione treatment resulted in decreased plasma resistin levels in type 2 diabetic patients [5]. Work to date on the metabolic effects of resistin has been complicated principally by several major caveats, including the lack of similarity between rodent and human resistin sequence [1,6] and site of production [7,8], the discrepancy between resistin mRNA and circulating protein levels [9,10] and alterations that occur in obesity [1,11–13] and whether resistin alters metabolism and insulin sensitivity in muscle or liver [14–21].

Nevertheless, It is clear from published literature that resistin may potentially play an important in vivo role in regulating carbohydrate and lipid metabolism. Reduced resistin levels via using an antisense approach [18], dominant inhibitory resistin [20] or knockout mice [15] were all associated with increased insulin sensitivity which typically manifested as decreased hepatic glucose production and lowered fasting blood glucose levels. Conversely, increasing resistin levels in transgenic mice [17,19], using recombinant protein [1,16,18] or surgically transplanting adipocytes overexpressing resistin [21] was associated with insulin resistance at the level of muscle or liver. Addition of the recombinant protein to cultured adipocytes [1], cardiomyocytes [22] or skeletal muscle cells [14,23] impaired insulin-stimulated glucose uptake and neutralization of resistin function with anti-resistin antibody improved insulin action in adipocytes [1]. Surprisingly little is known about the effect of resistin on fatty acid metabolism in skeletal muscle and it is imperative that we understand this given the well established role for fatty acids in regulating muscle insulin sensitivity [24]. Therefore, in this study we treated rat skeletal muscle cells with recombinant resistin and investigated changes in fatty acid uptake and metabolism and mechanisms underlying these processes.

2. Materials and methods

2.1. Materials

Cell culture medium (α-MEM) and all other cell culture components were purchased from Wisent (St Foy, QC, Canada), 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR) was purchased from Toronto Research Chemicals Inc (Toronto, Ont., Canada). Human insulin (Humulin®R) was from Eli Lilly (Toronto, Ont., Canada). [1-^{14}C] oleate was from Amersham (Baie d'Urfe, QC, Canada). Recombinant human resistin was obtained from Peprotech (Ottawa, Ont., Canada). BODIPY-conjugated palmitate was purchased from Molecular Probes (Eugene, OR). Antibodies for FATP-1, FATP-4, CD36, AMPKα-1 and AMPKα-2 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Polyclonal phosphospecific antibodies to AMPK (Thr-172) and ACC (Ser79) and anti-ACC antibody were from Cell Signaling (Beverly, MA). Oil red O and triethyl-phosphate were from Fluka Chemie (Buchs, Switzerland). All other reagents were of the highest grade available.

2.2. Cell culture

L6 rat skeletal muscle cells were cultured as described previously [25] in minimum essential medium (α-MEM) supplemented with 10% (v/v) fetal bovine serum and 1% antibiotic/antimycotic solution (100 units/ml penicillin, 100 μg/ml streptomycin, 250 ng/ml amphotericin B) in a humidified atmosphere of 95% air and 5% CO_2 at 37 °C. These stock cells were sub-cultivated before reaching confluence and standard growth medium was changed every two days. When required, cells were harvested with 0.25 mM trypsin and 0.2% EDTA (~1 min at 37 °C), resuspended with 10% or 2% medium for growth and differentiation, respectively, and seeded for the assigned experimental conditions.

*Corresponding author. Fax: +416 736 5698.
E-mail address: gsweeney@yorku.ca (G. Sweeney).

0014-5793/$30.00
doi:10.1016/j.febslet.2005.08.011

2.3. Measurement of fatty acid uptake

To determine fatty acid uptake, L6 skeletal muscle cells were grown on cover slips in 12-well plates. After 24 h treatment with or without resistin (50 nM), cells were starved for 3–5 h and incubated with insulin (100 nM) for 15 min, in the continued presence of resistin. After this incubation period, medium was aspirated and the cells were washed twice with PBS containing fatty acid-free albumin. Immediately after washing, the cells were incubated with BODIPY-conjugated palmitate (1 μM) for 2 min at 37 °C, then the cover slips were washed three times with cold PBS and transferred on mounted on clean glass slides using Dako antifade solution. Non-specific uptake is determined via competition with 5 mM palmitate. For confocal microscopy analysis, BODIPY-conjugated fatty acids were excited at 488 nm with Olympus 300 multiline argon laser. Fluorescent images were obtained using Fluoview software and fluorescence intensity quantitated by ImageJ software. Preliminary results using insulin identified that fluorescent fatty acid uptake occurred in a time- and concentration-dependent manner (data not shown).

2.4. Analysis of cell surface CD36 content

The level of CD36 at the cell surface was measured in intact cells by an antibody-coupled colorimetric assay. Briefly, L6 myoblast were grown in 12 well plates in the presence or absence of resistin (50 nM, 24 h) followed by the 5 h of serum starvation in the continued presence of resistin. Cells were then treated with or without insulin (100 nM) for 15 min. Subsequently, cells were quickly washed in ice-cold PBS and incubated with anti-CD36 polyclonal antibody (H300, Santa Cruz Biotechnology, 1:200 dilution) for 60 min at 4 °C. Cells were washed and fixed in 3% paraformaldehyde for 3 min on ice. The fixative was then neutralized by incubation in 10 mM glycine in ice-cold PBS for 10 min. Cells were blocked in 10% goat serum for 10 min and then incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG (1:1000 dilution, 4 °C) for 60 min. Cells were washed 5 times with ice-cold PBS and incubated for 30 min at room temperature with 1 ml of OPD reagent (0.4 mg ml ^{1}*O*-phenylenediamine di-hydrochloride and 0.4 mg ml^{-1} urea hydrogen peroxide in 0.05 M phosphate citrate buffer) per well. The reaction was stopped by adding 0.25 ml of HCl (3 M). The supernatant was collected and the absorbance was measured at 492 nm. Absorbance associated with non-specific binding (primary antibody omitted) was used as a blank.

2.5. Western blot analysis of fatty acid transporters

L6 cells were grown in 6-well plates and treated with or without resistin (50 nM) for 24 h, followed by serum-starving for 3–5 h in the continued presence of resistin and then cells were treated with insulin (100 nM). Plates were washed three times with ice-cold PBS then 200 μl of lysis buffer (135 mM NaCl, 1 mM $MgCl_2$, 2.7 mM KCl, 20 mM Tris (pH 8.0), Triton 1%, glycerol 10%, and protease and phosphatase inhibitors including 0.5 mM Na_3VO_4, 10 mM NaF, 1 μM leupeptin, 1 μM pepstatin, 1 μM okadaic acid, 0.2 mM PMSF) were added as previously described [26]. Whole cell lysate was centrifuged (12000 rpm, 4 °C for 10 min) and the supernatant was used for further analysis. An aliquot of the cell lysate was used to determine the protein concentration in each sample. Prior to loading onto SDS–PAGE gels, the samples were diluted 1:1 (v/v) with 2× Laemmli sample buffer (62.5 mM Tris–HCl (pH 6.8), 2% (w/v) SDS, 50 mM DTT, 0.01% (w/v) bromophenol blue). Aliquots of cell lysates containing 25 μg of protein were then subjected to SDS–PAGE (8–10% resolving gels), and then transferred to polyvinylidene difluoride (PVDF) membranes (Bio-Rad, Burlington, Ont., Canada). The expression level of AMPKα-1, AMPKα-2, ACC, FATP1, FATP4 and CD36 were determined using specific antibodies (1:500 dilution for AMPK-α1 and AMPK-α2, 1:1000 for all others). Phosphorylation level of AMPK (Thr-172) and ACC (Ser-79) was detected using phospho-specific antibodies at a 1:1000 dilution. Primary antibody detection was performed using horseradish peroxidase (HRP)-conjugated appropriate secondary antibody and visualized using enhanced chemiluminescence (Perkin–Elmer, Burlington, Ont., Canada).

2.6. Measurement of fatty acid oxidation

Fatty acid oxidation was measured by the production of $^{14}CO_2$ from [1-^{14}C] oleate as previously described [26] with a few modifications. Briefly, cells were cultivated in 60 × 15 mm Petri dishes with or without resistin (50 nM) for 24 h and starved for 3–5 h, in the continued presence of resistin, prior to addition of appropriate reagents for times and at concentration as indicated below. Cells were incubated for 2 h with medium containing 0.15 μCi ml^{-1} D-[1-^{14}C] oleate in the presence or absence of AICAR (1 mM) for 2 h. Each petri dish was sealed with parafilm which had a piece of Whatman paper taped facing the inside of the petri dish. After 2 h of incubation the Whatman paper was wet with 100 μl of phenylethylamine–methanol (1:1) to trap the CO_2 produced during the incubation period and 200 μl of H_2SO_4 (4 M) was then added.. After incubation for 1 h at 37 °C, the pieces of Whatman paper were removed and transferred to scintillation vials for radioactivity counting.

2.7. 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide assay

Cell viability was determined using the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT) assay (Sigma–Aldrich, St. Louis, MO). Briefly, cells were seeded at a density of 1×10^6 cells/ml in 96-well plates and incubated in the presence or absence of resistin (50 nmol/l) for 24 h. MTT was then added and the ability of cells to reduce this substrate to the blue formazan product was determined colorimetrically (550 nm) as an indicator of metabolically-active cells.

2.8. Oil red O staining of intracellular lipid

Determination of lipid content was carried out as described by the method of Pedrini et al. [27] with minor modifications. L6 myoblasts were grown on cover slips in 12 well plates and incubated with or without resistin (50 nM) for 24 h and serum starved 3–5 h in the continued presence of resistin. After this period, cells were fixed in 3.7% formaldehyde for 60 min and excess of formaldehyde was removed by three rinses in deionised water for 30 s. Subsequently, oil red O staining was carried out as described previously [28]. Images were obtained on a Laser Scanning Confocal Microscope (Olympus fluoview 300, 60× objective) using HeNe laser (Texas red excitation filter at 543 nm). The intensity of lipid droplets/cytoplasmic area was then analysed quantitatively by using Image-J software.

2.9. Statistical analysis

Data are expressed as means ± S.E.M. Statistical analysis was undertaken using paired Student's *t* test. Differences between groups were considered statistically significant when $P < 0.05$.

3. Results

We first examined the effect of resistin on BODIPY-conjugated palmitate uptake in L6 myoblasts. Fig. 1A shows fluorescence intensity in cells treated with resistin alone or in combination with insulin. It is clear that resistin decreased uptake of this long chain fatty acid whereas insulin caused the expected small increase in uptake. Quantitative analysis of this data shows that incubation of cells with resistin for 24 h significantly reduced both basal and insulin-stimulated fatty acid uptake (Fig. 1B). We demonstrated that the effect of resistin on fatty acid metabolism was not due to a non-specific effect on cell metabolism by conducting MTT assay under similar conditions and finding no change was induced by resistin (control 1.0 ± 0.02 and resistin (50 nM, 24 h) 1.03 ± 0.03). To further investigate the underlying mechanism for the decreased fatty acid uptake induced by resistin (Fig. 1) we next examined cell surface CD36 content and expression of fatty acid transporter proteins found in L6 myoblasts. Resistin decreased basal levels of cell surface CD36 and prevented insulin-induced CD36 translocation (Fig. 2A). Whereas no change in total expression of FATP-4 or CD36 was detected, we found that resistin significantly decreased expression of FATP-1 (Fig. 2B).

Having demonstrated that resistin decreased fatty acid uptake, we next examined if a similar effect on fatty acid oxidation was observed. To do this we measured $^{14}CO_2$ pro-

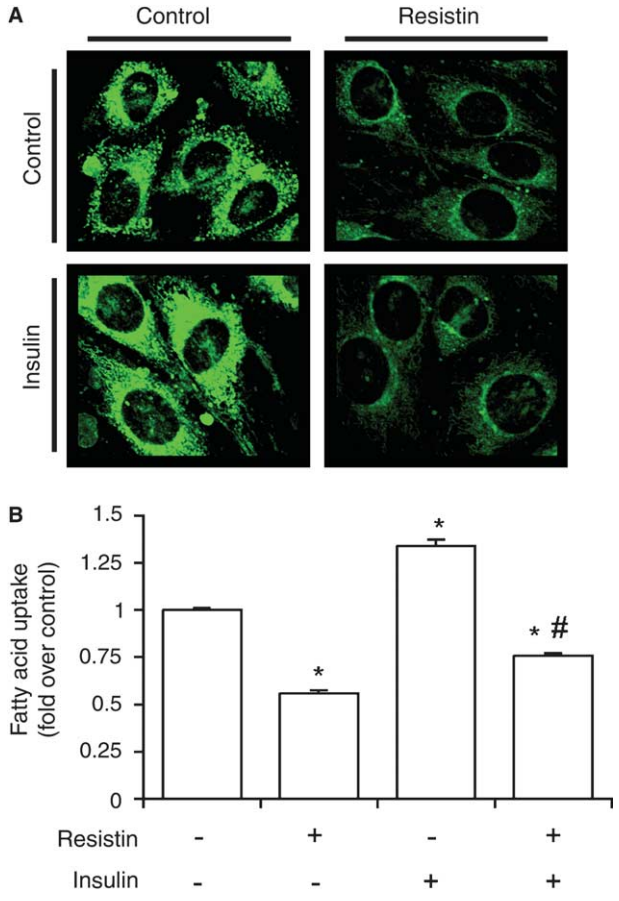

Fig. 1. Effect of resistin on uptake of BODIPY-palmitate. We examined uptake of the fluorescently labeled long chain fatty acid palmitate in L6 myoblasts. Representative images demonstrating decreased fatty acid uptake in cells treated with resistin (50 nM, 24 h) and increased uptake in response to insulin (100 nM, 15 min) are shown in A. Quantitative analysis of >25 individual cells from multiple fields of view is shown in B and values represent mean ± S.E.M. where * indicates $P < 0.05$ compared to control and # indicates $P < 0.05$ compared to resistin.

duction from [1-^{14}C] oleate and found that resistin treatment decreased fatty acid oxidation in L6 cells (Fig. 3). We used AICAR as a positive control to increase fatty acid oxidation levels and found that the ability of AICAR to increase fatty acid oxidation was also prevented by resistin (Fig. 3). We next investigated whether the effects of resitin on fatty acid uptake and metabolism were reflected in a change in intramuscular lipid accumulation. Oil red staining of myoblasts demonstrated that treatment of cells with resistin was associated with increased lipid staining (Fig. 4A) and this was found to be a statistically significant difference of almost 1.5-fold upon quantitative analysis (Fig. 4B).

Both AMPK and ACC play a critical role in regulating fatty acid oxidation, but it is not known whether 24 h treatment of muscle cells with resistin alters phosphorylation or expression of these key enzymes. We show in Fig. 5 that phosphorylation of AMPK is significantly reduced by resistin. However, no change in expression of either AMPK isoform (AMPK-α1 or AMPK-α2) was detected (Fig. 5). Similarly, we investigated the effect of resistin on ACC and found a decrease in phosphorylation (Fig. 6A) but no change in expression of ACC2, the isoform expressed in myoblasts (Fig. 6B).

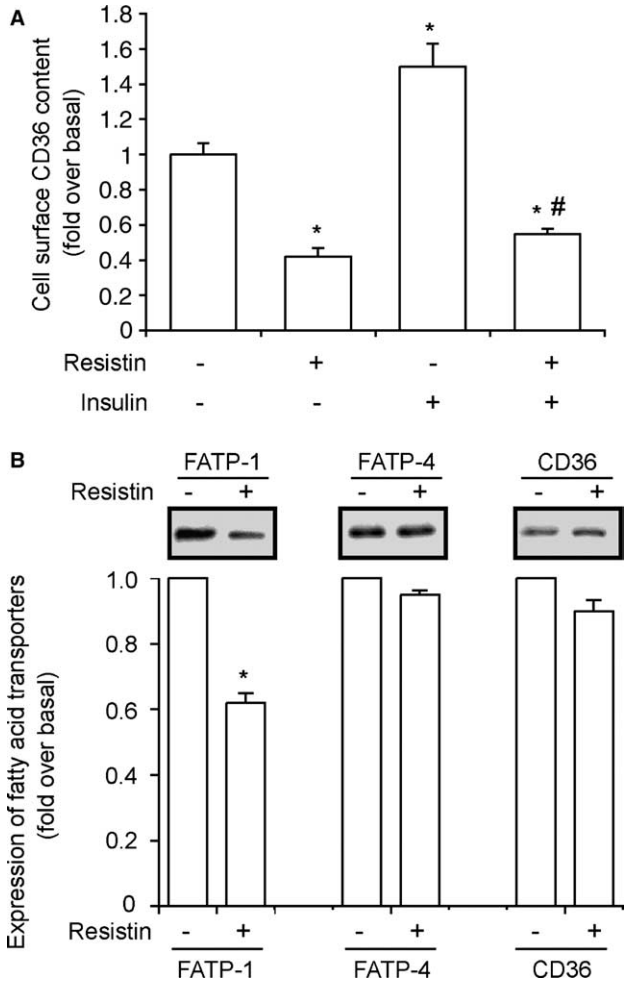

Fig. 2. Effect of resistin on cell surface CD36 levels and fatty acid transporter expression. The amount of CD36 detected at the cell surface was determined after treatment with insulin (100 nM, 15 min) in the presence or absence of resistin (50 nM, 24 h) as shown in A. In B, FATP-1, FATP-4 and CD36 expression in cells treated with resistin (50 nM, 24 h) were examined as described in Section 2. Representative images of individual experiments are shown together with quantitative analysis of three individual experiments where control is assigned a value of 1 and values represent mean ± S.E.M. In both A and B * indicates $P < 0.05$ compared to control and #$P < 0.05$ compared to insulin alone.

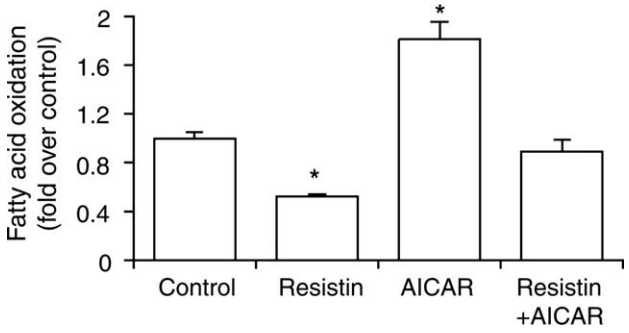

Fig. 3. Effect of resistin on fatty acid oxidation. The effect of resistin (50 nM, 24 h) on $^{14}CO_2$ production from [1-^{14}C] oleate is shown. AICAR (1 mM, 2 h) was also used as a positive control. Data are representative of at least three independent experiments expressed as mean ± S.E.M. and * indicates $P < 0.05$ compared to control.

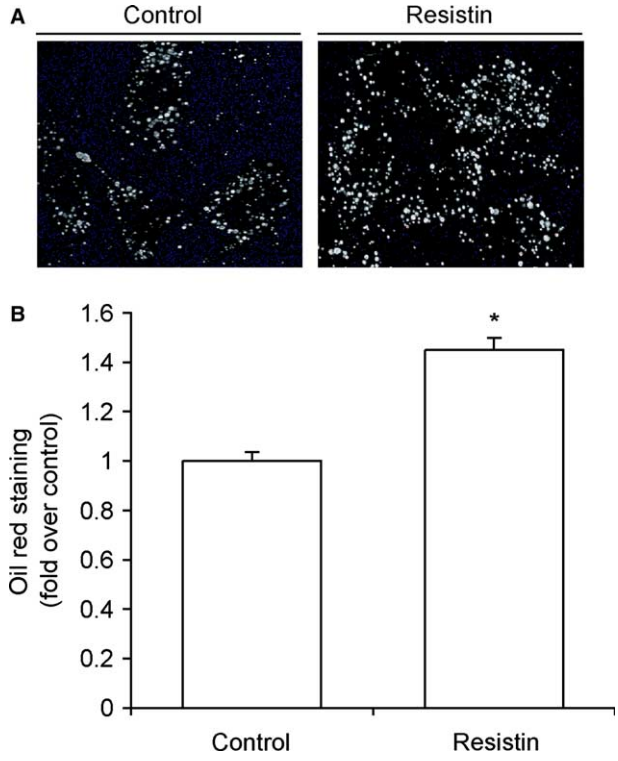

Fig. 4. Effect of resistin on lipid accumulation. Lipid accumulation in muscle cells was determined by oil red staining in control cells and cells which were treated with resistin (50 nM, 24 h). Representative images from analysis by confocal microscopy are shown in A while B shows a summary (mean ± S.E.M.) of quantitative analysis of >30 cells from each of two independent experiments and * indicates $P < 0.05$ compared to control.

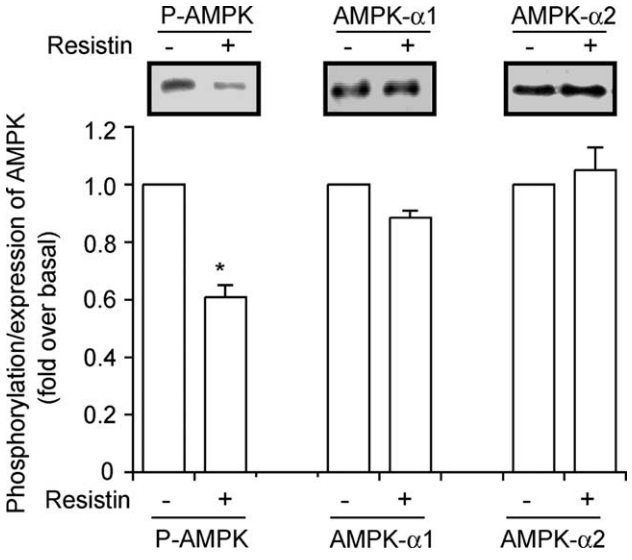

Fig. 5. Effect of resistin on AMPK phosphorylation and isoform expression. We examined AMPK phosphorylation and AMPK-α1 and AMPK-α2 content in lysates from cells treated with resistin (50 nM, 24 h) as described in Section 2. Representative images of individual experiments are shown together with quantitative analysis of three individual experiments where control is assigned a value of 1 and values represent mean ± S.E.M. where * indicates $P < 0.05$ compared to control.

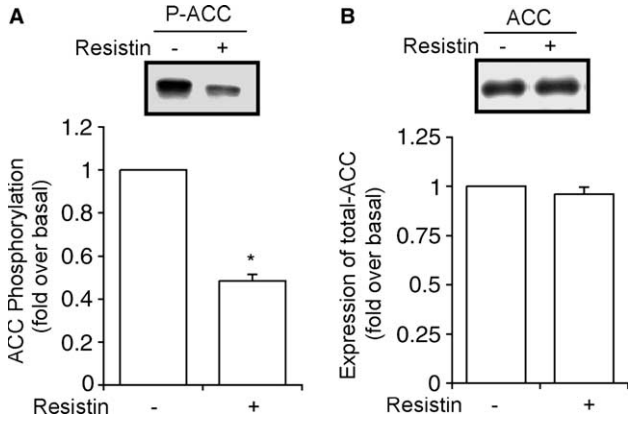

Fig. 6. Effect of resistin on ACC phosphorylation and expression. We examined ACC phosphorylation (A) and total ACC content (B) in lysates from cells treated with resistin (50 nM, 24 h) as described in Section 2. Representative images of individual experiments are shown together with quantitative analysis of three individual experiments where control is assigned a value of 1 and values represent mean ± S.E.M. where * indicates $P < 0.05$ compared to control.

4. Discussion

Several studies have proposed that endocrine effects of resistin may represent one mechanism underlying the development of skeletal muscle insulin resistance in obesity [2]. This resulted from initial studies where addition of the recombinant protein to normal mice or cultured adipocytes impaired insulin action and neutralization of resistin function with anti-resistin antibody improved insulin action in mice with diet-induced obesity [1]. More recently, preventing resistin action in transgenic mice expressing a dominant inhibitory version of the protein improved insulin sensitivity and glucose tolerance in mice [20]. It was speculated that this in vivo effect of resistin may involve decreased triglyceride and free fatty acid concentrations [20]. Despite the interest in resistin as a possible cause of insulin resistance in obesity, few studies which examine the metabolic effects of resistin have been completed. An ability to regulate glucose production in the liver [15–19], glucose uptake in adipocytes [4] and glucose uptake and metabolism in skeletal muscle [14,18,19,21,29] has been documented. However, the direct effect of resistin on fatty acid uptake and oxidation in skeletal muscle has not been determined.

In this study we show that chronic (24 h) treatment of L6 rat skeletal muscle cells with recombinant resistin decreased fatty acid uptake and metabolism. We have previously demonstrated that resistin can induce insulin resistance in skeletal muscle cells [14] and here we further investigated whether these effects of resistin manifested as an alteration in the total triacylglycerol accumulation in cells. We found increased lipid accumulation was induced by resistin, in keeping with the well characterized ability of intracellular lipid to induce insulin resistance. Interestingly, the bulk of available literature examining in vivo effects of resistin suggests that resistin regulates glucose homeostasis primarily by causing hepatic insulin resistance [15,16,18]. It is possible that our results correlate well with this hypothesis since decreased fatty acid uptake and utilization in skeletal muscle in vivo will likely lead to higher circulating free fatty acid levels. This elevated plasma free fatty acid level must be dealt with by other tissues and it is likely

that increased hepatic steatosis may be one result. Indeed, a very recent study has demonstrated that a decrease in circulating resistin levels in type 2 diabetic patients, induced by thiazolidinedione treatment, correlated with decreased fat content in liver and improved insulin sensitivity in this tissue [5]. Thus, we suggest it is possible that resistin may induce hepatic insulin resistance both by directly acting on hepatocytes or by altering the ability of skeletal muscle to contribute to fatty acid homeostasis.

Upon investigating mechanisms whereby resistin may control fatty acid metabolism we also demonstrated in this study that resistin decreased the level of AMPK phosphorylation in skeletal muscle, with no alteration in expression of either isoform. Similarly, in a previous in vivo study, phosphorylation of AMPK was attenuated in rats overexpressing resistin [19]. ACC phosphorylation and inactivation is directly mediated by AMPK [30,31]. Thus, a decrease in AMPK phosphorylation and activity would be expected to lead to decreased ACC phosphorylation, and thus increased activity of this enzyme. In keeping with this, we found that ACC phosphorylation was also decreased by resistin in this study. Increased ACC activity subsequently leads to increased malonyl co-A levels which mediate an inhibitory effect on CPT-1, preventing fatty acid transport into mitochondria. Therefore, this mechanism explains the observed decrease in fatty acid oxidation we observed in response to resistin. We did not find any change in ACC2 expression, the only isoform in L6 myoblasts and predominant form in rat skeletal muscle [31], in response to resistin. This is not surprising since alterations in the total amount of ACC in skeletal muscle do not seem to play an important role in regulating insulin resistance as they are unaltered by manipulations such as fasting and refeeding [32].

The mechanism whereby resistin alters AMPK phosphorylation is unknown. This is in part due to the fact that resistin receptors are yet to be cloned and we still do not have sufficient details on the signaling mechanisms regulated by resistin. Thus, although it is likely that crosstalk may occur between resistin-stimulated signaling pathways and components of the AMPK pathway, this has yet to be established. However, it is likely that the ability of resistin to decrease AMPK phosphorylation may be mediated by altering activity of upstream kinase or the phosphatases involved in deactivating AMPK. Phosphorylation of AMPK on Thr172 normally increases activity of the protein 50- to 100-fold [30] and LKB1 was recently identified as AMPK kinase [33]. However, whether resistin regulates LKB1 activity is at present unknown. Similarly, the ability of resistin to promote desphosphorylation of AMPK by PP2Cα, or the less potent PP2Ac [34] remains to be determined.

In summary, we have shown that resistin targets AMPK and regulates fatty acid uptake and metabolism in rat skeletal muscle cells. Specifically, resistin decreased both uptake and oxidation of long chain fatty acids. The mechanisms underlying these effects include decreased cell surface CD36 content and FATP-1 expression, a reduction in phosphorylation and activation of AMPK and a decrease in phosphorylation, therefore increased activity of ACC. Future work examining the effect of resistin on these parameters in skeletal muscle in vivo and the significance in the pathogenesis of insulin resistin will prove interesting.

Acknowledgements: Funding was provided by Natural Science and Engineering Research Council (NSERC) via a Discovery grant to GS and the Canadian Diabetes Association via a scholarship award to GS in honor of the late Mary A. Bodington. GS also acknowledges support of the Ontario Ministry of Economic Development and Trade via a Premiers Research Excellence Award. Funding from Canada Foundation for Innovation and Ontario Innovation Trust is also acknowledged.

References

[1] Steppan, C.M. et al. (2001) The hormone resistin links obesity to diabetes. Nature 409, 307–312.
[2] Steppan, C.M. and Lazar, M.A. (2004) The current biology of resistin. J. Intern. Med. 255, 439–447.
[3] Degawa-Yamauchi, M., Bovenkerk, J.E., Juliar, B.E., Watson, W., Kerr, K., Jones, R., Zhu, Q. and Considine, R.V. (2003) Serum resistin (FIZZ3) protein is increased in obese humans. J. Clin. Endocrinol. Metab. 88, 5452–5455.
[4] McTernan, P.G. et al. (2003) Resistin and type 2 diabetes: regulation of resistin expression by insulin and rosiglitazone and the effects of recombinant resistin on lipid and glucose metabolism in human differentiated adipocytes. J. Clin. Endocrinol. Metab. 88, 6098–6106.
[5] Bajaj, M., Suraamornkul, S., Hardies, L.J., Pratipanawatr, T. and DeFronzo, R.A. (2004) Plasma resistin concentration, hepatic fat content, and hepatic and peripheral insulin resistance in pioglitazone-treated type II diabetic patients. Int. J. Obes. Relat. Metab. Disord. 28, 783–789.
[6] Ghosh, S., Singh, A.K., Aruna, B., Mukhopadhyay, S. and Ehtesham, N.Z. (2003) The genomic organization of mouse resistin reveals major differences from the human resistin: functional implications. Gene 305, 27–34.
[7] Nagaev, I. and Smith, U. (2001) Insulin resistance and type 2 diabetes are not related to resistin expression in human fat cells or skeletal muscle. Biochem. Biophys. Res. Commun. 285, 561–564.
[8] Fain, J.N., Cheema, P.S., Bahouth, S.W. and Hiler, M. (2003) Lloyd Resistin release by human adipose tissue explants in primary culture. Biochem. Biophys. Res. Commun. 300, 674–678.
[9] Rajala, M.W. et al. (2004) Regulation of resistin expression and circulating levels in obesity, diabetes, and fasting. Diabetes 53, 1671–1679.
[10] Lee, J.H., Bullen Jr., J.W., Stoyneva, V.L. and Mantzoros, C.S. (2005) Circulating resistin in lean, obese and insulin-resistant mouse models: lack of association with insulinemia and glycemia. Am. J. Physiol. Endocrinol. Metab. 288 (3), E625–E632.
[11] Rajala, M.W., Lin, Y., Ranalletta, M., Yang, X.M., Qian, H., Gingerich, R., Barzilai, N. and Scherer, P.E. (2002) Cell type-specific expression and coregulation of murine resistin and resistin-like molecule-alpha in adipose tissue. Mol. Endocrinol. 16, 1920–1930.
[12] Steppan, C.M. and Lazar, M.A. (2002) Resistin and obesity-associated insulin resistance. Trends Endocrinol. Metab. 13, 18–23.
[13] Juan, C.C. et al. (2001) Suppressed gene expression of adipocyte resistin in an insulin-resistant rat model probably by elevated free fatty acids. Biochem. Biophys. Res. Commun. 289, 1328–1333.
[14] Moon, B., Kwan, J.J., Duddy, N., Sweeney, G. and Begum, N. (2003) Resistin inhibits glucose uptake in L6 cells independently of changes in insulin signaling and GLUT4 translocation. Am. J. Physiol. Endocrinol. Metab. 285, E106–E115.
[15] Banerjee, R.R. et al. (2004) Regulation of fasted blood glucose by resistin. Science 303, 1195–1198.
[16] Rajala, M.W., Obici, S., Scherer, P.E. and Rossetti, L. (2003) Adipose-derived resistin and gut-derived resistin-like molecule-beta selectively impair insulin action on glucose production. J. Clin. Invest. 111, 225–230.
[17] Rangwala, S.M., Rich, A.S., Rhoades, B., Shapiro, J.S., Obici, S., Rossetti, L. and Lazar, M.A. (2004) Abnormal glucose homeostasis due to chronic hyperresistinemia. Diabetes 53, 1937–1941.
[18] Muse, E.D., Obici, S., Bhanot, S., Monia, B.P., McKay, R.A., Rajala, M.W., Scherer, P.E. and Rossetti, L. (2004) Role of resistin in diet-induced hepatic insulin resistance. J. Clin. Invest. 114, 232–239.

[19] Satoh, H., Nguyen, M.T., Miles, P.D., Imamura, T., Usui, I. and Olefsky, J.M. (2004) Adenovirus-mediated chronic "hyper-resistinemia" leads to in vivo insulin resistance in normal rats. J. Clin. Invest. 114, 224–231.
[20] Kim, K.H., Zhao, L., Moon, Y., Kang, C. and Sul, H.S. (2004) Dominant inhibitory adipocyte-specific secretory factor (ADSF)/resistin enhances adipogenesis and improves insulin sensitivity. Proc. Natl. Acad. Sci. USA 101, 6780–6785.
[21] Kitagawa, Y. et al. (2004) Impaired glucose tolerance is accompanied by decreased insulin sensitivity in tissues of mice implanted with cells that overexpress resistin. Diabetologia 47, 1847–1853.
[22] Graveleau, C. et al. (2005) Mouse and human resistin impair glucose transport in primary mouse cardiomyocytes and oligomerization is required for this biological action. J. Biol. Chem., Epub June 27.
[23] Palanivel, R., Maida, A., Liu, Y. and Sweeney, G. (2005) Regulation of insulin signaling, glucose uptake and metabolism in rat skeletal muscle cells upon prolonged exposure to resistin. Diabetologia, in revision..
[24] Blaak, E.E. (2003) Fatty acid metabolism in obesity and type 2 diabetes mellitus. Proc. Nutr. Soc. 62, 753–760.
[25] Tajmir, P., Kwan, J.J., Kessas, M., Mozammel, S. and Sweeney, G. (2003) Acute and chronic leptin treatment mediate contrasting effects on signaling, glucose uptake, and GLUT4 translocation in L6-GLUT4myc myotubes. J. Cell Physiol. 197, 122–130.
[26] Ceddia, R.B., Somwar, R., Maida, A., Fang, X., Bikopoulos, G. and Sweeney, G. (2005) Globular adiponectin increases GLUT4 translocation and glucose uptake but reduces glycogen synthesis in rat skeletal muscle cells. Diabetologia 48, 132–139.
[27] Pedrini, M.T., Kranebitter, M., Niederwanger, A., Kaser, S., Engl, J., Debbage, P., Huber, L.A. and Patsch, J.R. (2005) Human triglyceride-rich lipoproteins impair glucose metabolism and insulin signalling in L6 skeletal muscle cells independently of non-esterified fatty acid levels. Diabetologia 48, 756–766.
[28] Koopman, R., Schaart, G. and Hesselink, M.K. (2001) Optimisation of oil red O staining permits combination with immunofluorescence and automated quantification of lipids. Histochem. Cell Biol. 116, 63–68.
[29] Pravenec, M. et al. (2003) Transgenic and recombinant resistin impair skeletal muscle glucose metabolism in the spontaneously hypertensive rat. J. Biol. Chem. 278, 45209–45215.
[30] Hardie, D.G. (2004) The AMP-activated protein kinase pathway – new players upstream and downstream. J. Cell Sci. 117, 5479–5487.
[31] Munday, M.R. (2002) Regulation of mammalian acetyl-CoA carboxylase. Biochem. Soc. Trans. 30, 1059–1064.
[32] Winder, W.W., MacLean, P.S., Lucas, J.C., Fernley, J.E. and Trumble, G.E. (1995) Effect of fasting and refeeding on acetyl-CoA carboxylase in rat hindlimb muscle. J. Appl. Physiol. 78, 578–582.
[33] Hawley, S.A., Boudeau, J., Reid, J.L., Mustard, K.J., Udd, L., Makela, T.P., Alessi, D.R. and Hardie, D.G. (2003) Complexes between the LKB1 tumor suppressor, STRAD alpha/beta and MO25 alpha/beta are upstream kinases in the AMP-activated protein kinase cascade. J. Biol. 2, 28.
[34] Davies, S.P., Helps, N.R., Cohen, P.T. and Hardie, D.G. (1995) 5′-AMP inhibits dephosphorylation, as well as promoting phosphorylation, of the AMP-activated protein kinase. Studies using bacterially expressed human protein phosphatase-2C alpha and native bovine protein phosphatase-2AC. FEBS Lett. 377, 421–425.